

Homeologous Recombination Plays a Major Role in Chromosome Rearrangements That Occur During Meiosis of *Brassica napus* Haploids

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ABSTRACT

Chromosomal rearrangements can be triggered by recombination between distinct but related regions. *Brassica napus* (AACC; $2n = 38$) is a recent allopolyploid species whose progenitor genomes are widely replicated. In this article, we analyze the extent to which chromosomal rearrangements originate from homeologous recombination during meiosis of haploid *B. napus* ($n = 19$) by genotyping progenies of haploid \times euploid *B. napus* with molecular markers. Our study focuses on three pairs of homeologous regions selected for their differing levels of divergence (N1/N11, N3/N13, and N9/N18). We show that a high number of chromosomal rearrangements occur during meiosis of *B. napus* haploid and are transmitted by first division restitution (FDR)-like unreduced gametes to their progeny; half of the progeny of *Darmor-bzh* haploids display duplications and/or losses in the chromosomal regions being studied. We demonstrate that half of these rearrangements are due to recombination between regions of primary homeology, which represents a 10- to 100-fold increase compared to the frequency of homeologous recombination measured in euploid lines. Some of the other rearrangements certainly result from recombination between paralogous regions because we observed an average of one to two autosyndetic A–A and/or C–C bivalents at metaphase I of the *B. napus* haploid. These results are discussed in the context of genome evolution of *B. napus*.

ACCUMULATING evidence indicates that chromosomal rearrangements are commonplace within and among species and that they contribute to the evolution, adaptation, and speciation of plants and animals (STEBBINS 1971; RIESEBERG 2001; COGHLAN *et al.* 2005). For instance, deletions and duplications modify gene copy number, which can induce change in gene expression (SONG and MESSING 2003) and phenotype (GUO *et al.* 1996; VEITIA 2005). In plants, changes in chromosome and genome organization are prompted by interspecific hybridization and/or polyploidization (WENDEL 2000). Studies of young natural and experimentally synthesized interspecific hybrids (RIESEBERG *et al.* 1995; BAACK *et al.* 2005) and polyploids (SONG *et al.* 1995; FELDMAN *et al.* 1997; LUKENS *et al.* 2006) have revealed that extensive and diverse genomic changes can arise immediately at the onset of genome merging or within a few generations. Subse-

quent rearrangements, which occur over longer periods of time, have been less extensively characterized even if they induce changes in karyotype and chromosome organization (LYSAK *et al.* 2006) that blur the initial hybridization/polyploidization events (WOLFE 2001) and lead to genome downsizing (LEITCH and BENNETT 2004). Most rearrangements occur at meiosis and can be mediated by the homologous recombination pathway. Deletions, duplications, and translocations can then arise when information for the repair of double-strand breaks (DSBs) is taken from (i) the same chromosome (“intrachromosomal recombination,” DEVOS *et al.* 2002; CHANTRET *et al.* 2005), (ii) elsewhere in the genome (“ectopic recombination,” PETROV *et al.* 2003), or (iii) homeologous chromosomes in polyploids and interspecific hybrids (“homeologous recombination,” UDALL *et al.* 2005). Homeologous exchanges have been shown to drive introgressive hybridization in *Gossypium* (CRONN *et al.* 2003), to be responsible for the adaptation of *Helianthus* haploid species to new environments (RIESEBERG *et al.* 2003), and to increase the range of genetic variation

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observed for important ecological and agronomic traits like flowering time (PIRES *et al.* 2004) or seed yield (OSBORN *et al.* 2003) in newly synthesized *Brassica napus*.

This study focuses on the oilseed crop (*B. napus*; AACC, $2n = 38$), which is a young allopolyploid species resulting from multiple independent hybridization events between ancestors of the modern diploid *B. oleracea* (CC, $2n = 18$) and *B. rapa* (AA, $2n = 20$) (U 1935; PALMER *et al.* 1983; SONG and OSBORN 1992). It is now largely accepted that the diploid progenitors of *B. napus* are widely replicated although it is still not clear whether they have evolved from a hexaploid ancestor or via segmental duplication of one or two ancestral genomes (TRUCO *et al.* 1996; PARKIN *et al.* 2003, 2005; LUKENS *et al.* 2004; LYSAK *et al.* 2005). Most genomic segments and genes of the *B. napus* genome are found in multiple copies, two of which are primary homeologues (regions from the A and C genomes that share the most recent common ancestry), while the remaining ones are paralogous to one or the other of these copies. Alignment of the A- (N1–N10) and C-genome (N11–N19) linkage groups allows identification of regions of primary homeology on the basis of the longest stretches of collinearity (PARKIN *et al.* 2003). It is noteworthy that the degree of conserved synteny varies considerably among linkage groups, with some stretches of collinear marker loci extending to entire linkage groups (*e.g.*, N1 and N11) while others extend only to half-linkage groups (*e.g.*, N9 and N18). Identification of regions of primary homeology is confirmed by the localization of homeologous recombination events, resulting in *de novo* homeologous nonreciprocal translocations (HNRT) (PARKIN *et al.* 1995; SHARPE *et al.* 1995), the latter called homeologous nonreciprocal transposition by UDALL *et al.* (2005) with the same acronym. HNRT events have been reported by these authors at frequencies of 0.43–1.6% of total recombination events in several mapping populations of euploid *B. napus* (AACC, $2n = 38$); the highest frequencies were observed when resynthesized *B. napus* was used as a parent. Preexisting reciprocal (LOMBARD and DELOURME 2001; OSBORN *et al.* 2003; PIQUEMAL *et al.* 2005) and nonreciprocal homeologous translocations (UDALL *et al.* 2005) have also been identified among different accessions of *B. napus* and shown to stimulate further rearrangements in their vicinities.

B. napus haploids (AC, $n = 19$) are attractive models with which to study the rate and pattern of homeologous recombination. First, these plants undergo a complete meiosis with variable and sometimes huge amounts of chromosome pairing at metaphase I (MI) (OLSSON and HAGBERG 1955; RENARD and DOSBA 1980; ATTIA and RÖBBELEN 1986; JENCZEWSKI *et al.* 2003). Second, the extent of affinity between the homeologous chromosomes/regions of *B. napus* is revealed since there is no pairing between homologues. Third, these plants pro-

duce viable restituted gametes with somatic chromosome numbers (MORINAGA and FUKUSHIMA 1933; TAI and IKONEN 1988), which is crucial for the occurrence and transmission of intergenomic exchanges. Finally, it should be noted that haploid plants arise spontaneously within natural populations at a frequency up to 6/1000 (THOMPSON 1969; STRINGHAM and DOWNEY 1973).

In this article, we analyze the extent and pattern of chromosomal rearrangements that occur during meiosis of *B. napus* haploids (AC, $n = 19$), with particular attention to the role of homeologous recombination. Our study focuses on three pairs of homeologous regions (N1/N11, N3/N13, and N9/N18) chosen to be representative of the different levels of synteny and homeologous recombination (SHARPE *et al.* 1995; PARKIN *et al.* 2003, 2005; UDALL *et al.* 2005). Our study shows that recombination preferentially occurs between regions of primary homeology and suggests that it may also occur between others regions showing intragenomic or intergenomic homology.

MATERIALS AND METHODS

Plant material: The production of haploid plants from *B. napus* cv. *Darmor-bzh* and *Yudal* has been described by JENCZEWSKI *et al.* (2003). Haploid plants were used as female parents with the male euploids donating haploid pollen. A first F₁ population was produced by crossing 5 haploids of *Darmor-bzh* with 1 euploid plant of *Yudal* (DxHz F₁ population, 172 plants). A second F₁ population was obtained by crossing 9 haploids of *Yudal* with 1 euploid plant of *Darmor-bzh* (YxHz F₁ population, 126 plants). Progeny were named according to their population with *x* designating the number of the maternal haploid plant and *z* designating the offspring number. One and 2 plants were chosen in the YxHz (Y9H11) and the DxHz (D11H29, D11H2) F₁ populations, respectively, and backcrossed to their recurrent parent (*i.e.*, *Yudal* when the parental haploid originated from *Darmor-bzh* and reciprocally). Each of these BC₁ populations contained a minimum of 85 plants. Additionally, a doubled haploid line of *B. oleracea*, *HDEM*, and a *B. napus* cv. *Darmor-bzh* euploid line were used as controls for BAC–FISH analysis.

Cytology: Flow cytometry was performed on 170 and 123 plants from the DxHz and the YxHz F₁ populations, respectively, to assess chromosome number with an accuracy of plus or minus two chromosomes according to EBER *et al.* (1997).

For meiotic analyses, floral buds were fixed in Carnoy's solution (ethanol:chloroform:acetic acid, 6:3:1) for 24 hr and stored in 50% ethanol. Observations on pollen mother cells (PMCs) were performed on anthers squashed and stained in a drop of 1% acetocarmine solution; 37 PMCs were observed at MI to establish the average meiotic behavior of the *Darmor-bzh* haploid at this stage. A minimum of 10 PMCs per plant were observed at MI to verify the chromosome numbers of 59 F₁ and 5 BC₁ plants.

FISH with a genome-specific BAC clone: FISH analyses were carried out on mitotic chromosomes of *B. oleracea* cv. *HDEM* and *B. napus* cv. *Darmor-bzh* and on anther meiocytes of the *Darmor-bzh* haploid at MI. Mitotic metaphase preparation from seedling root tips was as described by SNOWDON *et al.* (1997). Meiotic chromosome preparation was the same as for mitotic observation. FISH was performed as extensively described in LEFLON *et al.* (2006). Briefly, BAC clone

BoB014O06 was labeled by random priming with biotin-14-dUTP (Invitrogen, San Diego; Life Technologies) and used as a probe at 100 ng/slide. Fluorescence images were captured using a CoolSnap HQ camera (Photometrics, Tucson, AZ) on an Axioplan 2 microscope (Zeiss, Oberkochen, Germany) and analyzed using MetaVue (Universal Imaging, Downingtown, PA).

Molecular markers: DNA extraction was performed as described by LOMBARD and DELOURME (2001) on a subset of 117 and 103 plants from the DxHz and the YxHz F₁ populations, respectively; these all display $2n = 38$ chromosomes (flow cytometry estimates confirmed by direct chromosome counting on a subset of plants). DNA extraction of backcross populations was performed on young leaves as described by MORALES *et al.* (2005), using a Microlab Star Hamilton robot. All the markers used in this study have been previously placed on a reference map constructed using doubled haploid progeny of *Darmor-bzh* and *Yudal* euploid parents (LOMBARD and DELOURME 2001; DELOURME *et al.* 2006). Linkage groups N1, N3, and N9 represent chromosomes from the A genome of *B. napus* while groups N11, N13, and N18 represent chromosomes from the C genome of *B. napus*.

RAPD markers (one-letter prefix) were described by FOISSET *et al.* (1996). Microsatellite markers originated either from the BBSRC (prefixed with Na, Ol, or Ra; LOWE *et al.* 2004) or from the CELERA consortium (prefixed with CB, BRAS, or MR; PIQUEMAL *et al.* 2005). PCR-specific markers derived from *Arabidopsis* are described by SILLITO *et al.* (2000) or FOURMANN *et al.* (2002). Physical functional markers (PFM) are prefixed CZ and have been developed from coding sequence of *Arabidopsis* and Brassica ESTs (H. BELGRAM and B. CHALHOUB, unpublished data; primer sequences are available upon request to Genoplante). SINE markers (prefixed with JLP) have been described in PRIETO *et al.* (2005).

PCR assays were conducted essentially as described in the quoted articles. Forward primers (5') were tailed with M13 to be revealed by LICOR technology. PCR products were analyzed on a 5.5% acrylamide gel and dye detected with LICOR as described by PIQUEMAL *et al.* (2005), on a 2.5% agarose gel as described by FOISSET *et al.* (1996), or on a 16-capillary ABI Prism 3130xl as described by ESSELINK *et al.* (2004).

Determination of allele copy number: Quantitative fluorescent-PCR analysis was used to determine the allele copy number of the haploid parents. PCR products were analyzed by capillary electrophoresis sequencer. Data were collected by Gene Scan v3.1 software and analyzed with GeneMapper v3.7 (Applied Biosystems, Foster City, CA). Peak height and area of alleles from multiallelic markers were estimated using GeneMapper. For haploid progeny alleles peak height and area ratios were shown to be highly correlated for some markers.

For each locus, there were three groups of plants, one with $r = 0$ copies of the haploid parent (HP) allele, one with $r = 1$ copy of the HP allele, and one with $r = 2$ copies of the HP allele. Plants with no peak associated with the HP allele were directly considered to satisfy $r = 0$. However, a more refined statistical analysis was needed to discriminate between plants where a peak was present ($r = 1$ or $r = 2$). For each locus, preliminary graphical representations of the data showed a clear linear relationship between the peak areas of the diploid and haploid parent alleles, with two distinct slopes corresponding to the cases $r = 1$ and $r = 2$. After these preliminary investigations, the following model was considered for each locus,

$$Y_i = r \cdot \beta \cdot X_i + \varepsilon_i, \quad (1)$$

where Y_i is the peak area of the HP allele measured on plant i , X_i is the peak area of the diploid parent (DP) allele on plant i , β is the basic regression coefficient, and, for each plant, $r = 1$

with probability p or $r = 2$ with (lower) probability $1 - p$. The error term ε_i was assumed to follow a normal distribution with standard error equal to $\sigma_0 + \gamma X_i$. The four unknown parameters β , p , σ_0 , and γ of the model were estimated by maximum likelihood, separately for each locus. In statistical terms the model (1) is a mixture of two regression models with probabilities p and $1 - p$, where the components correspond to case $r = 1$ or $r = 2$. Once the parameters of such a model are estimated, it is possible to estimate the probability p_i that each plant i satisfies $r = 1$, given the data and the Y_i -value for that plant (EVERITT and HAND 1981; JENCZEWSKI *et al.* 2003). The odds ratio $p_i/(1 - p_i)$ is the estimated ratio between the probabilities that plant i satisfies $r = 1$ or $r = 2$. For subsequent data analyses, a plant i was considered to have one copy of the HP allele ($r = 1$) when its odds ratio was >100 . It was considered to have two copies of the HP allele when its odds ratio was $<1/100$. In the few intermediate cases (0–8% of plants depending on marker), information was considered insufficient to decide allele copy number and hence they were discarded from further analyses. This analysis was carried out for every plant in the DxHz population for loci on N1, N9, and N11 and in the progeny of *Yudal* haploids for loci on N3.

All statistical analyses were performed with R language on R version 2.2.1 software (R DEVELOPMENT CORE TEAM 2005) or on S-Plus 2000 software.

RESULTS

In situ hybridization survey of meiosis in haploid

***B. napus*:** FISH analysis was first carried out using BoB014O06 to probe mitotic chromosomes of *B. oleracea* cv. *HDEM* and *B. napus* cv. *Darmor-bzh*. Only the nine C genome chromosomes in *B. napus* were labeled by this probe; however, the hybridization signal is not homogeneous along the chromosomes (Figure 1, a and b). At meiosis, MI chromosomes of haploid *Darmor-bzh* were labeled with the BoB014O06 probe to determine the occurrence of autosyndesis (A–A or C–C pairing) *vs.* allosyndesis (A–C pairing) (Figure 1, d, f, and h). Autosyndesis was shown to be commonplace at MI in *Darmor-bzh* haploids since 84% of the 49 observed PMCs contained at least one autosyndetic bivalent. Having classified chromosome configurations (univalent, I; bivalent, II; trivalent, III; and tetravalent, IV) and determined the genome origin of each chromosome, we calculate the average meiotic behavior of the *Darmor-bzh* haploid at MI to be: $3 I^A + 2.2 I^C + 5.2 II^{C-A} + 0.8 II^{A-A} + 0.7 II^{C-C} + 0.1 III + 0.04 IV$. Autosyndetic bivalents therefore represented 22% of the bivalents observed. The multivalent configurations observed also included intragenomic pairing.

Genomic structure of plants in the progeny of haploid *B. napus*: Flow cytometry showed that the vast majority of plants in the progeny of haploid *B. napus* had an estimated chromosome number ranging from 37 to 39 (Figure 2). Interestingly, 10 plants (3%) appear to be triploid (AAACCC) because they display an estimated chromosome number distributed around 57; these estimates were confirmed by direct cytological chromosome counting for 4 plants. To further corroborate the

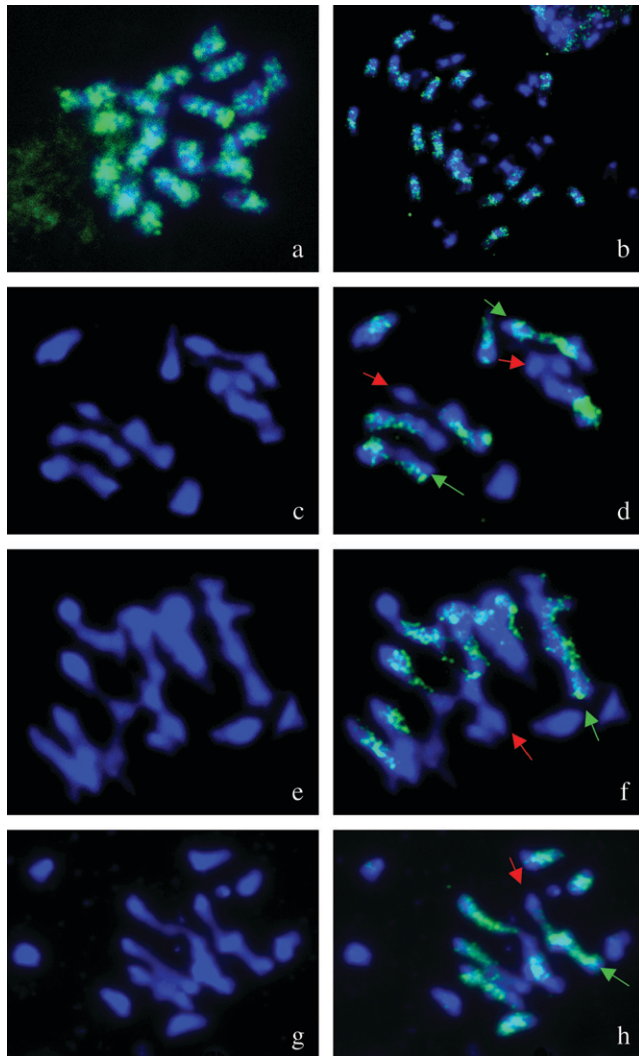


FIGURE 1.—Detection of autosyndesis at metaphase I using FISH on pollen mother cells from haploid *Darmor-bzh*. Mitotic chromosomes of *B. oleracea* cv. *HDEM* (a) and *B. napus* cv. *Darmor-bzh* (b) are probed with BAC BoB014O06 (green) and counterstained with DAPI (blue), to show that this BAC stains only C genome chromosomes. DAPI staining is then used on anther meiocytes to establish the meiotic behavior of every *Darmor-bzh* PMC at MI (c, e, and g) and then combined with BoB014O06 FISH signals (d, f, and h) to distinguish autosyndetic and allosyndetic associations. Red and green arrows indicate autosyndetic bivalents between A and C chromosomes, respectively. (d) Seven bivalents including one ring bivalent (four autosyndetic and three allosyndetic) and five univalents. (e) Seven bivalents (two autosyndetic and five allosyndetic) and five univalents. (f) Six bivalents (two autosyndetic and four allosyndetic) and seven univalents.

flow cytometry results, we directly counted the number of chromosomes of a subset of 55 plants with an estimated $2n = 38$ chromosomes. Only 1 plant had 39 chromosomes while the remaining 54 had 38 as expected (Figure 2). Extending this estimate to the entire population, we predict that 77–93% of progeny plants of *Darmor-bzh* and *Yudal* haploids have 38 chromosomes. Ninety percent of these 54 plants displayed >75% of

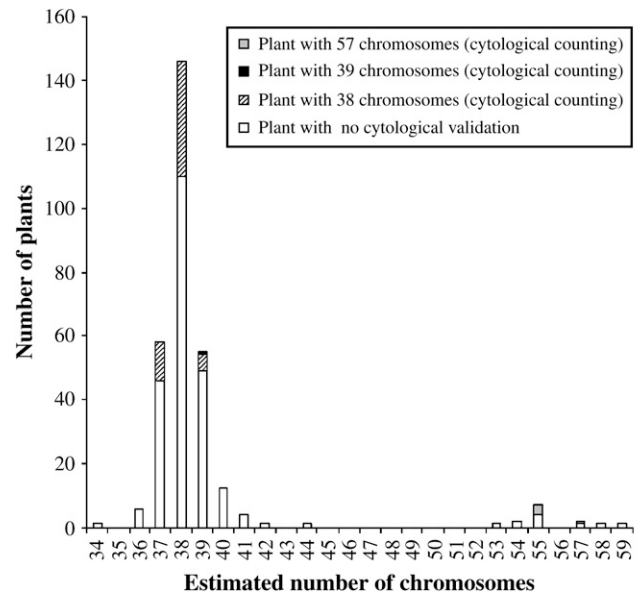


FIGURE 2.—Analysis of chromosome number in the progenies of *B. napus* haploids. Flow cytometry was used to estimate the chromosome number for 293 haploid-derived offspring. Cytological observations at MI of meiosis on a mean number of 17 PMCs per plant were performed on 55 plants selected to have chromosome estimates ranging from 37 to 39 (open and solid bars).

PMCs with 19 bivalents at MI, which demonstrates that the $2n = 38$ plants originated from restituted female gametes that had one copy of each of the 19 chromosomes of the haploid mother plants. Most viable gametes produced by haploid plants were therefore analogous to first division restitution (FDR) gametes.

Detection of homeologous exchanges with PCR molecular markers: In F_1 plants ($2n = 38$) derived from these FDR-like gametes all the markers from the HP are expected to be present when no recombination occurs during HP meiosis (Figure 3, offspring A). Reciprocally, HNRTs, which originate from disjunction of reciprocal homeologous exchanges, can be detected in the progeny of haploid \times euploid *B. napus* by looking for plants that lack the HP alleles for a given set of loci and had duplicate HP alleles at corresponding homeologous loci (Figure 3, offspring D and E). In this study, we analyzed transmission and/or duplication of molecular markers spanning three pairs of homeologous regions that represent a range of synteny levels (described in PARKIN *et al.* 2003, 2005) and homeologous recombination rates in euploid lines (SHARPE *et al.* 1995; UDALL *et al.* 2005): (i) the bottom sections of linkage groups N3 and N13 are structurally very different and do not recombine with one another, (ii) group N18 is collinear with one end of group N9 and they display moderate levels of homeologous recombination with each other, and (iii) groups N1 and N11 are syntenous along their entire length and display the highest frequencies of HNRTs.

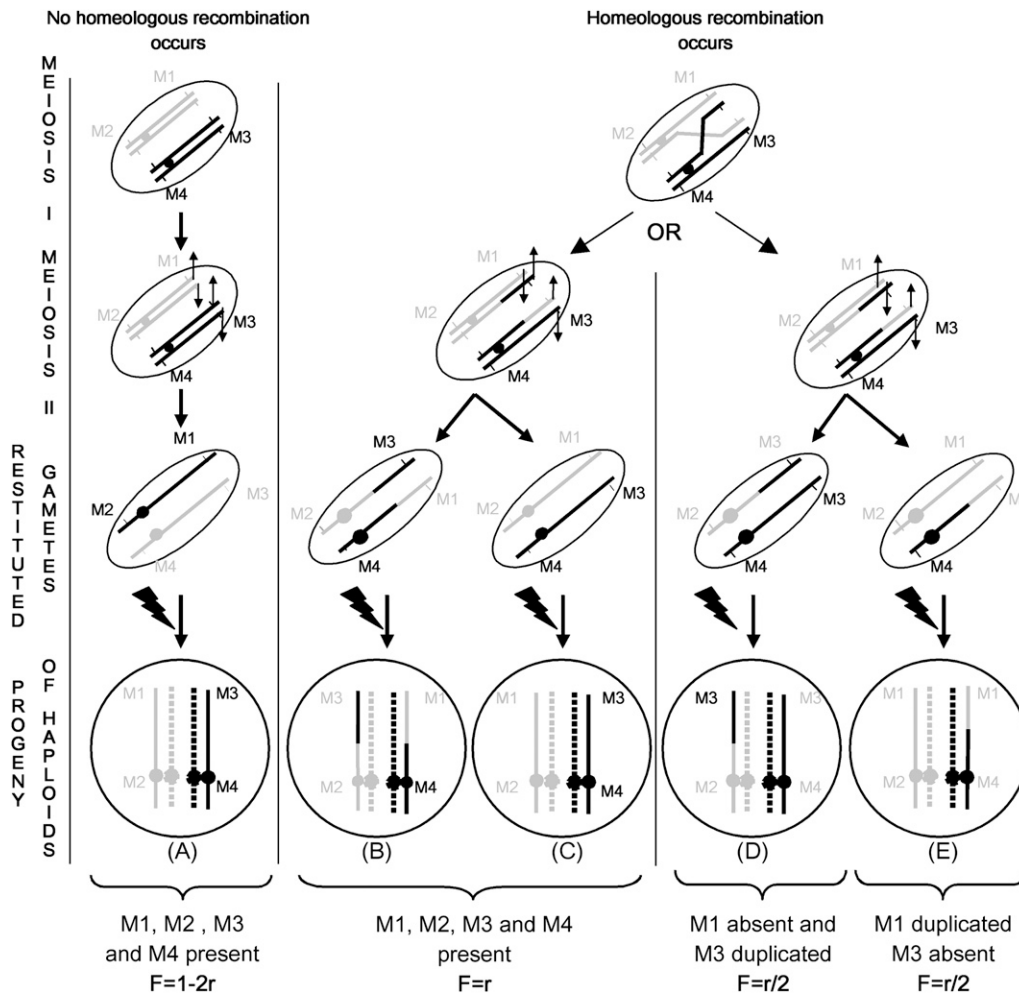


FIGURE 3.—Segregation of molecular markers in the progenies of *B. napus* haploids. This chromatid model of homeologous exchanges assumes that *B. napus* haploids produce restituted gametes with 19 chromosomes. When no homeologous recombination occurs during meiosis of the haploid, then all markers from the haploid parent (HP) are transmitted to its progeny (offspring A). When homeologous recombination occurs during meiosis of the haploid parent, recombinant chromatids may either (i) move to the same pole of anaphase, meaning all the markers from the HP are transmitted to its progeny (offspring B and C) despite the presence of a homeologous reciprocal translocation (HRT), or (ii) move to opposite anaphase poles, meaning some of the markers from the HP are not transmitted to its progeny while others, carried by the (partially homeologous) substituting chromosomal segment are duplicated (offspring D and E). These latter exchanges are commonly termed homeologous nonreciprocal translocations (HNTRs). r , frequency of homeologous recombination; jagged arrowhead, fertilization by male gametes ($n = 19$) produced by the euploid male parent.

changes are commonly termed homeologous nonreciprocal translocations (HNTRs). r , frequency of homeologous recombination; jagged arrowhead, fertilization by male gametes ($n = 19$) produced by the euploid male parent.

HP allele loss in the progeny of *B. napus* haploids:

We analyzed transmission of 20 molecular markers spanning the six linkage groups described above. We genotyped the haploid parents and their progeny with codominant markers to be sure that absence of HP alleles was due to nontransmission of markers. The proportion of plants in the progeny of *Darmor-bzh* haploids that lacked HP alleles (*e.g.*, Figure 4) varied from 0 to 14%, depending upon the locus (Table 1). The highest frequencies were obtained for markers located at opposing ends of N11. We confirmed that markers located on the bottom section of N3 rarely displayed loss of HP alleles by genotyping an additional 103 progeny of *Yudal* haploids with the same markers; it was observed that only one plant (Y9H11) lacked the HP alleles for marker IH08a (Table 1). Looking at all progeny plants in all but three cases offspring that lacked the HP allele at one or more loci on a linkage group displayed HP allele(s) present at other loci on the same linkage group (Table 2); this demonstrates that nontransmission of the HP allele was not due to the whole-chromosome loss.

Duplicated HP alleles in the progeny of *B. napus* haploids:

Detection of HP allele duplication (*e.g.*, Figure 5) was achieved for four markers that revealed six loci in regions homeologous to segments where missing HP alleles had been identified (Table 1). Exact correspondence between homeologous loci was ascertained when marker assays detected multiple loci mapping to collinear blocks (O112F11a/b, CB10081a/b). When these markers were not available, we used markers anchored in *Arabidopsis* to find the most likely homeologues within collinear blocks (data not shown). The proportion

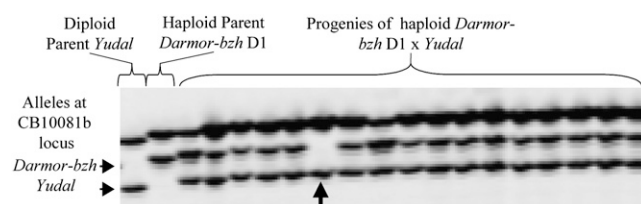


FIGURE 4.—Inheritance of the HP allele at CB10081b in the F₁ progeny of the *Darmor-bzh* haploid, D1. One plant lacks the *Darmor-bzh* HP allele (arrow).

TABLE 1
Loss and duplication of haploid parent (HP) alleles at corresponding homeologous loci in progeny plants

Haploid parent	Loss of haploid parent (HP) alleles					Duplication of HP alleles at homeologous loci				
	LG	PI	Loci	No. of progeny lacking HP allele	% of progeny lacking HP allele	LG	PI	Loci	No. of progeny with duplicated HP allele	% of progeny with duplicated HP allele
<i>Darmor-bzh</i>	N1	0	CB10081b	10	9	N11	0.02	CB10081a	7	6
		0.41	O112F11b	5	4		0.43	O112F11a	5	4
		0.66	Bras026	1	1		—	—	—	—
	N11	0.02	CB10081a	16	14	N1	0	CB10081b	13	11
		0.24	CB10587	14	13		—	—	—	—
		0.43	O112F11a	11	10		0.41	O112F11b	9	8
		0.56	Na12C08	2	2		—	—	—	—
		0.83	CZ4A12840b	14	12		—	—	—	—
	N9	0.09	CZ0B687858	2	2	N18	—	—	—	—
	N18	0.03	CB10092	4	6	N9	—	—	—	—
		0.39	JLP042	9	8		0.09	CZ0B687858	9	10
		1	Bras031	0	0		—	—	—	—
	N3	0.28	Bras029	1	1	N13	—	—	—	—
		0.65	IH08a	0	0		0.85	JLP011	0	0
		0.97	CZ4A27950	1	1		—	—	—	—
N13	0.85	JLP011	5	4	N3	—	—	—	—	
<i>Yudal</i>	N3	0.28	Bras029	0	0	N13	—	—	—	—
		0.65	IH08a	1	1		0.85	JLP011	0	0
		0.97	CZ4A27950	0	0		—	—	—	—
	N13	0.85	JLP011	0	0	N3	—	—	—	—

LG, linkage group; PI, position index, relative position of loci on linkage group calculated by dividing the genetic position of loci by the size of the LG on the reference map: 0 designates the top of the linkage group and 1 the bottom. Markers appearing on the same line are exact homeologues or located in homeologous regions.

of plants in the progeny of *B. napus* haploids having two copies of HP alleles varied from 0 to 11.5% depending on both the linkage group and the position of the markers (Table 1). The highest frequencies were obtained for markers located at the top of N1 and N9 while the lowest was at JLP011, located at the distal end of N13 (Table 1).

Concurrent loss and duplication of homeologous HP alleles by HNRTs: Simultaneous analysis of homeologous loci showed that nontransmission of HP alleles was frequently associated with the duplication of HP alleles in homeologous regions (Table 2). For example, 14 of 19 plants lacked *Darmor-bzh* alleles at CB10081a and/or O112F11a on N11 and had two copies of *Darmor-bzh* alleles at homeologous loci CB10081b and/or O112F11b on N1 (Table 2, events A–H). Taking into account that H has two independent rearrangements, 20 and 7 instances of concurrent loss and duplication of linked loci were observed for N1/N11 and N18/N9, respectively. Subsequent analysis of other loci on the same linkage groups revealed that concurrent loss and duplication were rarely limited to interstitial loci (Table 2, events F–H) and usually extended to the end of linkage groups (Table 2, events A–E, H–K, and A'). Six

plants had a pattern of duplication and loss that was compatible with the occurrence of two distinct simultaneous rearrangements on a single chromosome (Table 2, events B, E, F, H, and J). Finally, we observed over twice as many plants with simultaneous loss of HP alleles on N11 and duplication on N1 than the number of plants with concurrent loss of HP alleles on N1 and duplication on N11 (14 *vs.* 6; Table 2, events A–H *vs.* H–K).

In summary, concurrent loss and duplication of homeologous alleles represented 51% of events for N1/N11 and 64% for N18/N9 (Table 2), indicating that approximately half the chromosomal rearrangements we detected are HNRTs. These HNRTs can be verified by checking the inheritance of HP alleles in controlled backcross progeny.

Independent loss and duplication of homeologous HP alleles: Both loss without duplication and duplication without loss was observed on N1/N11 and N18/N9. Likewise, the only plant (Y9H11) that lacked a HP allele at a N3 marker did not display HP allele duplication on the homeologous region of N13. We observed twice as many instances of loss without duplication than duplication without loss on N1/N11, but similar levels of these two kinds of rearrangements on N18/N9 (Table 2). The

TABLE 2
Chromosomal rearrangements detected by simultaneous analysis of loss and duplication of haploid parent (HP) alleles at different homeologous loci

Categories	N11					N1			Event name	No. of plants	Total no. of plants
	CZ4A12840b	Na12C08	O112F11a	CB10587	CB10081a	Bras026	O112F11b	CB10081b			
Concurrent loss and duplication									A	4	19
				ND					B	1	
				ND					C	1	
									D	1	
									D	2	
									E	1	
									F	2	
									G	1	
									H	1	
									I	2	
									J	1	
									K	2	
	Loss without duplication									L	2
									M	2	
									N	2	
									O	2	
									P	1	
									Q	1	
									R	1	
									S	1	6
Duplication without loss									T	1	
									U	1	
									V	1	
									W	1	
									X	1	
									Y	3	9
Unknown ^a									Z	6	

(continued)

TABLE 2
(Continued)

Categories	N18		N9: <i>CZ0B687858</i>	Event name	No. of plants	Total no. of plants
	Bras031	JLP042				
Homeologous loss–duplication events detected on N9/N18						
Concurrent loss and duplication				A'	2	7
				B'	3	
		ND		C'	2	
Loss without duplication				E'	1	2
		ND		F'	1	
Duplication without loss				D'	2	2
				G'	1	
Unknown ^a				H'	2	3
N13: <i>JLP011</i>						
Homeologous loss–duplication events detected on N3/N13						
Loss without duplication	Bras029	IH08a	CZ4A27950	A''	1	1
				B''	1	
Unknown ^a		ND		C''	5	5

Loci in *italics* indicate that the number of copies of HP alleles has been estimated; loci not in *italics* were used to analyze transmission of HP alleles but not the number of copies. Solid cells indicate two copies of HP alleles, shaded cells indicate at least one copy, and open cells designate that HP alleles are absent. Two adjacent markers on a linkage group showing a different copy number (present/absent; one copy/two copies) indicate a chromosomal rearrangement. Six data points are missing (ND); hence complete estimates of rearrangement cannot be determined for these plants.

^aPlants in which copy number of HP alleles at homeologous loci is unknown.

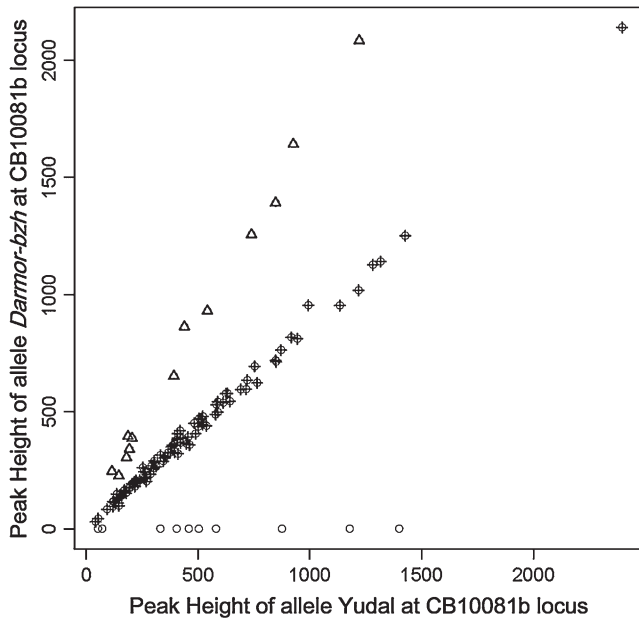


FIGURE 5.—Determination of copy number of the *Darmor-bzh* HP allele at CB10081b. A quantitative value for the *Yudal* allele peak area (*x*-axis) was used as a baseline to which the *Darmor-bzh* allele peak area (*y*-axis) has been compared by maximum-likelihood analyses: O, plants with no *Darmor-bzh* allele at the CB10081b locus (loss); +, plants with a single *Darmor-bzh* allele at the CB10081b locus (no duplication, no loss); Δ, plants with two *Darmor-bzh* alleles at the CB10081b locus (duplication).

number and position of independently duplicated or lost loci were variable and nonrandom since independent loss and duplication of HP alleles occurred more frequently at the distal ends of linkage groups than interstitially (Table 2, events U, X, and A'').

Characterizing chromosomal rearrangements by backcrossing: Two plants D1H29 (Table 2, event D) and D11H2 (Table 2, event A') that displayed simultaneous loss and duplication of *Darmor-bzh* alleles at homeologous loci on N1/N11 and N9–N18, respectively, were backcrossed to *Yudal* to analyze marker segregation on implicated chromosomes (Figure 6).

The first plant, D1H29 had a pattern of duplication and loss that was compatible with the presence of a translocation of N1 (duplicated) on N11 (substituted) [N11.N1 (T)]. Using molecular markers distributed along these groups, we observed that markers located at the distal end of N11 did not display the *Darmor-bzh* allele while three markers from the corresponding part of N1 were duplicated (Figure 7A). The latter markers segregated at 3:1 in the (D1H29 × *Yudal*) backcross population but at 1:1 in another BC₁ progeny that was free from any [N11.N1 (T)]. We then analyzed the extent of linkage disequilibrium between markers flanking the breakpoints of this putative HNRT (Brass084, O112F11b, and CB10081b on N1 and Na12C08 on N11) and observed that these markers cosegregated more often than expected by chance ($\chi^2 < 0.01\%$; data not

shown). These results confirmed that D1H29 carried a distal [N11.N1 (T)] HNRT (Figure 7A). Estimating the size of genetic intervals carrying the breakpoints of this HNRT was not straightforward. Figure 6A assumes that no further recombination event took place in this interval during the backcrossing process. In reality recombination is likely to have occurred and considerable thought must go into detecting these events when analyzing marker segregation data. For example, if one were to look for backcross recombination events purely by looking at presence of M3^D CB10081b and absence of M2^D Na12C08 without considering the segregation of other markers, one-quarter of backcross progeny (group 3) could be misclassified as having recombined during backcrossing. Fortunately all recombination between [N11.N1 (T)] and N1 and half of recombination between [N11.N1 (T)] and N11 can be detected by combining allelic composition and cosegregation analysis (Figure 6B: plants with unexpected cosegregation pattern 01 in group 1, 10 in groups 2 and 4, and 11 in group 3). Overall, 13 of 81 plants were detected as recombinant in the [N11.N1 (T)] interval of D1H29, which represents a map distance of 15 cM. Allele composition information also identifies 5 additional plants (highlighted by arrows in Figure 6) that displayed abnormal allelic composition: 2 plants lacked one locus CB10081a and 3 had an extra copy of the CB10081b locus. Among these 5 outliers, 2 plants were aneuploid ($2n = 37$ and $2n = 39$) while the remaining 3 had a very irregular meiotic behavior with a high number of either univalents (1 plant, 60% of PMCs) or multivalents (2 plants, 75 and 50% of PMCs).

The same analyses were performed on D11H2 and confirmed that this plant carried a distal HNRT of N9 on N18 [N18.N9 (T)] (Figure 7B). As allele composition information could not be determined for our markers on N9 and N18, the size of this HNRT was determined using cosegregation patterns 01 and 00 that can be unambiguously recognized as recombinant or not recombinant, respectively. We thus estimated that 5 cM lay between W11.560 and CZ0B687858 in the interval carrying the N18.N9 (T) breakpoint of D11H2 (Figure 7B).

Finally we tried to characterize the rearrangement carried by plant Y9H11 that lacked *Yudal* alleles at 3 loci on N3 and did not display two copies of the HP allele at 9 loci in its homeologous region on N13 (Figure 7C). Using 13 markers from N3, we demonstrated that the missing segment was interstitial and encompassed a region between 28 cM (12% of linkage group length) and 34 cM (24%) on N3, depending on breakpoint positioning (Figure 7C). We then tested for marker duplication on other regions displaying intragenomic (N5, N1) or intergenomic (N11) homology to the missing part of N3. Part of N15 is also paralogous to the missing part of N3; however, polymorphic markers could not be found and hence this possibility remains unexamined. Allelic segregation ratios for markers on N1 and N11 did

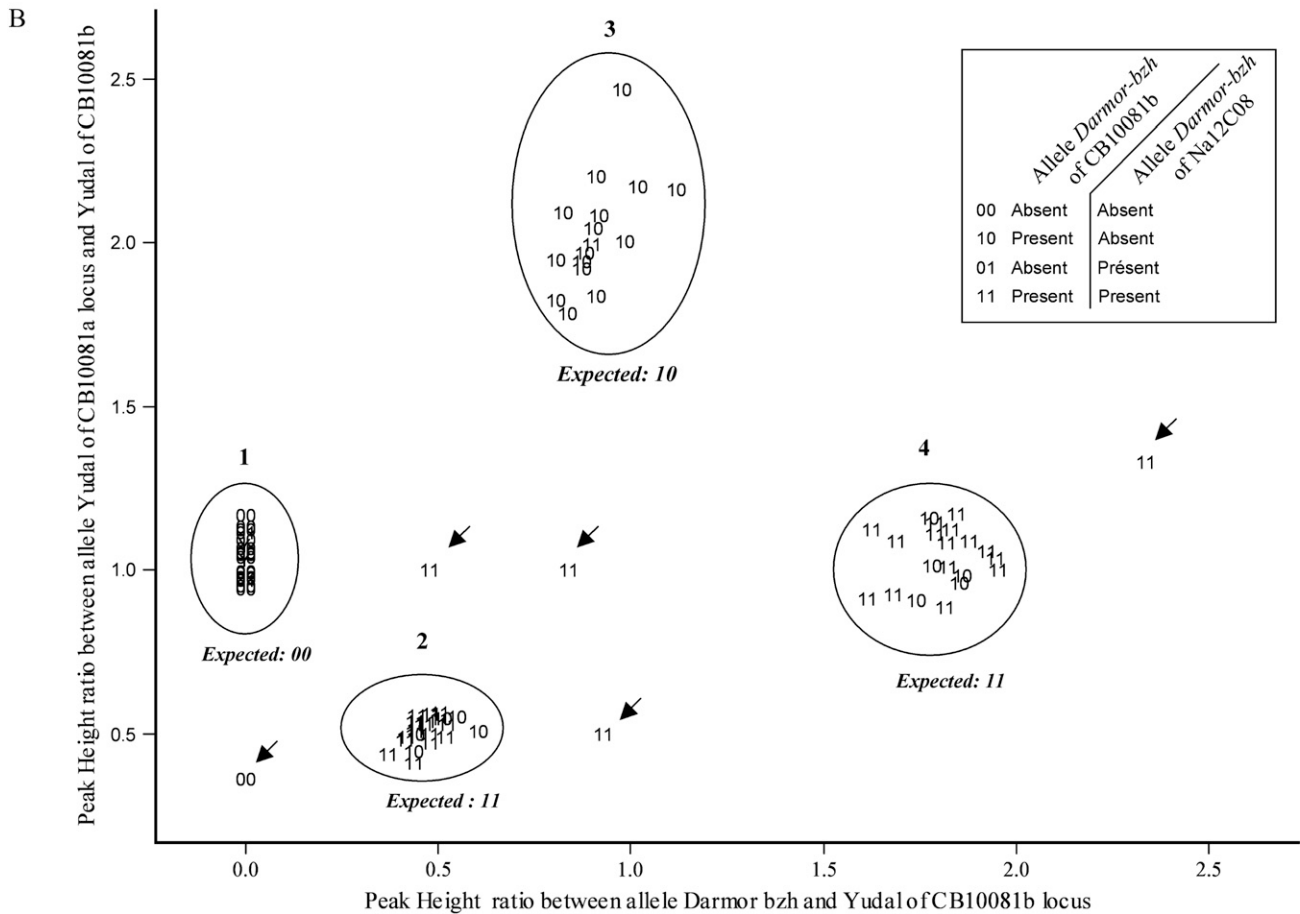
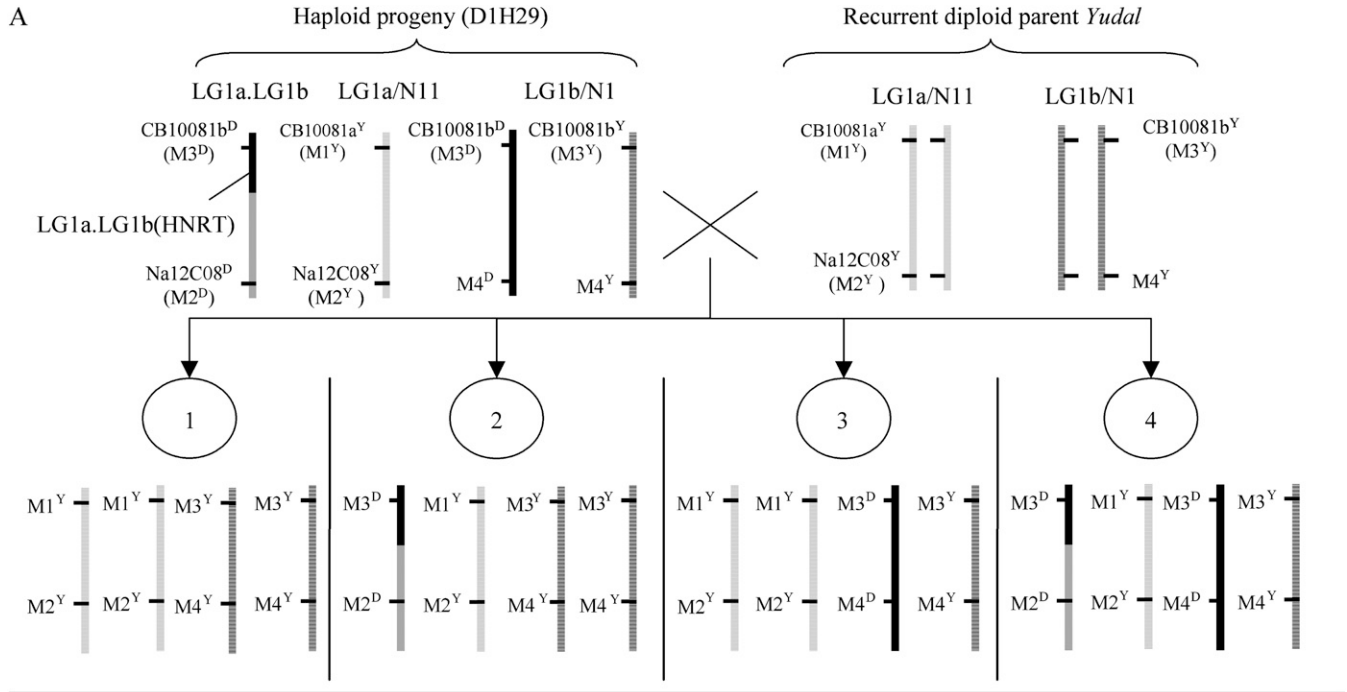


FIGURE 6.—Expected and observed segregation of the N11.N1 homeologous nonreciprocal translocation in the backcross progeny of D1H29. (A) Expected segregation of the N11.N1 HNRT assuming no further recombination event in the genetic intervals carrying the breakpoints of this HNRT. Three markers are considered: M1, CB10081a; M2, Na12C08; and M3, CB10081b. D1H29 lacks the *Darmor-bzh* allele at CB10081a (M1^D) and had two copies of the *Darmor-bzh* allele at CB10081b (M3^D). Chromatid sorting results in four groups of BC₁ offspring that can be unambiguously recognized by comparing allelic composition of *Darmor-bzh* and

not deviate from the 1:1 ratio, indicating absence of duplication. In contrast, a marker on N5 segregated 3:1 (excess of *Yudal* allele) but no evidence that this locus cosegregated with CZ5b711213 on N3 (Figure 7C) was found, indicating that this region of N5 is duplicated but has not been translocated onto N3.

Comparison of recombination rates between homeologous and homologous: Considering that a minimum of 50% of the detected losses of HP alleles resulted from crossovers between homeologous regions and that half the products of homeologous recombination can be detected in the progenies of *B. napus* haploids (Figure 3), we compared the frequencies of homeologous recombination measured in this study to the frequencies of homologous recombination estimated for the same intervals from the genetic mapping data. The ratio between the proportions of homeologous and homologous recombinants was lower for two intervals on N1 (CB10081b–OI12F11b) and N18 (JLP042–Bras031) because the corresponding markers were genetically independent on the linkage groups (Table 3). Notwithstanding this point, we observed that this ratio averaged a constant 10-fold decrease of homeologous *vs.* homologous recombination for most intervals and linkage groups, with the notable exception of N11, which showed a higher relative proportion of homeologous recombinants (Table 3).

We also took advantage of the fact that our approach was similar to that of UDALL *et al.* (2005), by comparing the frequency of HNRTs generated per chromosome arm during meiosis of haploid *vs.* euploid lines. We observed that the frequency of homeologous recombination was increased 10- to 100-fold during meiosis of *B. napus* haploids when compared to that of the natural or synthetic euploid *B. napus* (Table 4). This ratio varied considerably among the linkage groups, the highest increase in the rate of homeologous recombination in haploid *vs.* euploid lines being observed at the top of N18–N9.

DISCUSSION

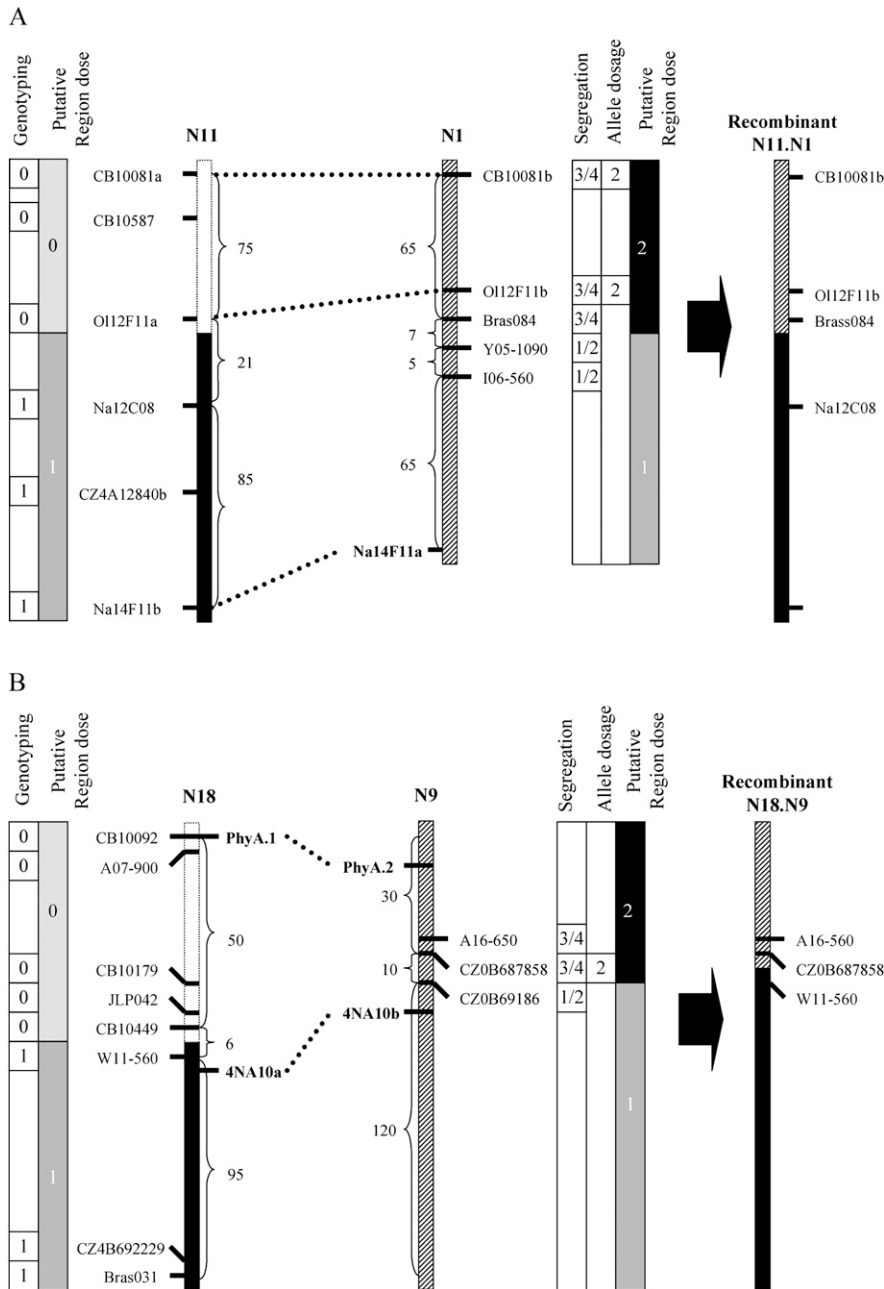
In this study, we have analyzed in progeny of haploid plants ($2n = 19$) transmission and/or duplication of molecular markers spanning three pairs of homeologous regions (N1/N11, N3/N13, and N9/N18) that represented a range of levels of synteny and homeolo-

gous recombination. We have shown that duplication and/or loss of chromosomal regions frequently occurs during meiosis of *B. napus* haploids and that half of the changes are due to recombination between regions of primary homeology. We also observed an unexpected number of autosyndetic bivalents, which strongly suggests that recombination can also occur between paralogous regions, leading to part of chromosomal rearrangements. These results raise the question of the role of haploids in the long-term evolution of the *B. napus* genome.

High occurrence and transmission of homeologous recombination products during meiosis of *B. napus* haploids: Meiosis and gamete production in haploid forms of *B. napus* has been well documented (MORINAGA and FUKUSHIMA 1933; OLSSON and HAGBERG 1955; RENARD and DOSBA 1980; ATTIA and RÖBBELEN 1986; TAI and IKONEN 1988). Our study moves two steps further in demonstrating that (i) homeologous exchanges occur during meiosis of *B. napus* haploids and that (ii) recombined chromosomes can be transmitted to the progeny of *B. napus* haploids by unreduced FDR-like gametes.

We have first proved that concurrent loss and duplication of homeologous loci can be used to detect *de novo* homeologous nonreciprocal translocations (HNRTs), also called homeologous nonreciprocal transposition by UDALL *et al.* (2005), by demonstrating in two plants that the haploid parent alleles located in the vicinity of the missing part of chromosomes no longer segregated independently from those that are duplicated on the corresponding homeologous region. This is due to the fact that translocations create “recombined linkage groups” that usually display reduced recombination in the vicinity of exchange breakpoints (SYBENGA 1975; PARKER *et al.* 1982), therefore promoting linkage disequilibrium between markers initially carried by different linkage groups. Assuming that simultaneous loss and duplication of linked loci in one plant originated from the same homeologous exchange, we detected a total of 18 distal and 7 interstitial HNRTs resulting from single and double homeologous crossovers, respectively (Table 2). These HNRTs represent approximately half the rearrangements we detected. We also observed patterns of loss and duplication that are compatible with occurrence of three crossovers spanning over two homeologous regions (Table 2, events B, E, F, H,

Yudal alleles at CB10081a and CB10081b ($M1^Y/M3^D/M3^Y$). If the cosegregation pattern of the *Darmor-bzh* alleles at CB10081b ($M3^D$) and Na12C08 ($M2^D$) is used, groups 1 and 3 can be identified by absence–absence (00) and presence–absence (10), respectively; however, groups 2 and 4 cannot be distinguished using these two marker alleles. (B) Observed segregation. Physical location on this plot reveals the four expected groups of BC_1 offspring (circled) from analysis of copy number ratios at CB10081a and CB10081b loci. Five outlying individuals (arrows) display abnormal allelic composition at CB10081a and/or CB10081b. Each plant is symbolized by its cosegregation pattern for two *Darmor-bzh* alleles at CB10081b and Na12C08 loci, respectively. The expected cosegregation pattern in the absence of recombination at the Na12C08–CB10081b interval is indicated above each of the four circles. Plants that display discrepancies between the expected cosegregation pattern (00 in group 1, 01 in group 3, and 11 in groups 2 and 4) and that observed are considered recombinant at the Na12C08–CB10081b interval. Plants in agreement with expected copy number ratios are considered nonrecombinant.



and J). All these results indicate that crossovers frequently and preferentially occur between homeologous chromosomes during meiosis of *B. napus* haploids. Therefore a large proportion, if not all, of the bivalents observed at MI, which appear alike, are in fact chiasmatic.

Our results also confirm what MORINAGA and FUKUSHIMA (1933), OLSSON and HAGBERG (1955), and TAI and IKONEN (1988) had previously observed, *i.e.*, that most of the functional and viable gametes produced by *B. napus* haploid contain one copy of each of the 19 chromosomes of *B. napus* and are therefore genetically similar to first division restitution gametes. Our study is the first to demonstrate that recombined chromosomes can be transmitted by these FDR-like

restituted gametes, allowing the immediate restoration of euploid chromosome number ($2n = 38$) and fertility (data not shown) in the progenies of haploids. However, our results indicate that HNRTs can promote abnormal segregation of chromosomes in subsequent generations, probably by inducing multivalent formation (SYBENGA 1975; RAMSEY and SCHEMSKE 2002); indeed, 6–28% of PMCs observed at MI in the progeny of haploids carrying HNRTs displayed quadrivalents (D11H2 and D1H29 revealed approximately one-quarter of PMCs with quadrivalents). Disjunction of these multivalents can lead to further rearrangements or to aneuploidy, as probably exemplified by the occurrence of five plants in the D1H29 backcross progeny with abnormal allelic composition (Figure 6).

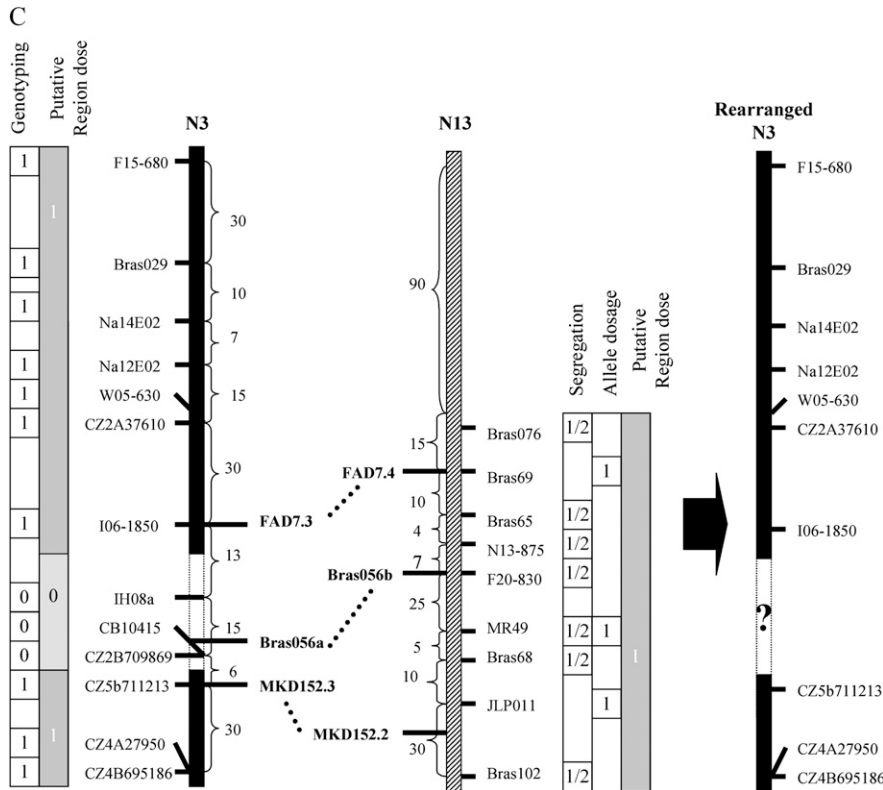


FIGURE 7.—Continued.

Frequency of homeologous recombination is greatly enhanced in *B. napus* haploids: Quantifying the number of concurrent losses and duplications of HP alleles can be used to estimate the proportion of chromosomal rearrangements transmitted by FDR-like gametes that

are due to homeologous recombination. However, this approach leads to underestimating the rate of homeologous recombination. Meiotic homeologous exchanges lead both to homeologous reciprocal translocations (HRTs) and to HNRTs (Figure 3), but only the latter

TABLE 3

Comparison of the rate of homologous *vs.* homeologous recombination measured on the same intervals

Linkage groups	Locus interval	% homeologous recombination	% homologous recombination ^a	Ratio ^{b,c}
N11	CB10081a–CB10587	7	22	0.3**
	CB10587–OI12F11a	11	15	0.7
	OI12F11a–Na12C08	8	15	0.5*
	Na12C08–CZ4A12840b	12	15	0.8
N1	CB10081b–OI12F11b	6	46	0.1**
	OI12F11b–Bras026	3	11	0.2**
N18	CB10092–JLP042	5	20	0.2**
	JLP042–Bras031	8	42	0.2**
N3	CZ4A27950–IH08a	1	20	0.1**
	IH08a–Bras029	1	50	0**

^aThe proportion of homologous recombination at each location was obtained by analyzing the DH mapping population used to build the reference map.

^bThis ratio, which reflects the rate of decrease of homeologous *vs.* homologous recombination, is estimated by dividing the percentage of homeologous recombination by the percentage of homologous recombination for the same interval.

^c χ^2 -tests were performed to compare the relative proportion of homologous and homeologous recombinants at each location: ** $P < 1\%$; * $P < 5\%$.

TABLE 4
Comparison of *de novo* viable HNRT frequencies generated during meiosis of haploid *vs.* euploid lines

Linkage group carrying the HNRT	Origin of duplicated segment	Progenies (female × male)	Interval observed	No. of <i>de novo</i> HNRTs detected	Mean no. of <i>de novo</i> HNRTs per plant	Ratio ^{a,b}
Top N1	Top N11	Haploid × euploid	CB10081b–Brass026	6	0.052	12**
		Euploid × euploid (UDALL <i>et al.</i> 2005)	pW169d–pX128e	6	0.009	
Top N11	Top N1	Haploid × euploid	CB10081a–Na12C08	14	0.121	32**
		Euploid × euploid (UDALL <i>et al.</i> 2005)	pW125c–pC128g	5	0.008	
Top N18	Top N9	Haploid × euploid	CB10092–JLP042	7	0.080	106**
		Euploid × euploid (UDALL <i>et al.</i> 2005)	pW123d–pW227	1	0.002	
Bottom N3	Bottom N13	Haploid × euploid	Brass29–Cz4A27950	0	0.000	—
		Euploid × euploid (UDALL <i>et al.</i> 2005)	pW147b–pX136a	0	0.000	—

^a This ratio, which reflects the rate of increase of haploid *vs.* euploid homeologous recombination, is estimated by dividing twice the mean number of *de novo* HNRTs per plant detected in the haploid × euploid progenies by the mean number of *de novo* HNRTs per plant detected in the euploid × euploid progenies (data from UDALL *et al.* 2005). Magnification of the numerator by a factor of 2 aims at compensating for the presence of two homologous chromosomes in the euploids while there is only one in the haploids.

^b χ^2 -tests were performed to compare the relative proportion of homologous and homeologous recombinants at each location: ** $P < 1\%$; * $P < 5\%$.

can be detected in the progeny of *B. napus* haploids. Likewise the patterns of segregation described in Figure 3 do not take into account the occurrence of trivalents and quadrivalents that represent 5–10% of associated chromosomes per MI (JENCZEWSKI *et al.* 2003). Disjunction in these multivalents can generate gametes carrying uncoupled losses and duplications despite the occurrence of recombination between regions of primary homeology. Finally, homeologous recombination may generate lethal or deleterious chromosomal assortments that cannot be detected in the progenies of haploids because the individuals derived from these gametes, or the gametes themselves, do not survive or are counterselected. In that respect, HRTs and HNRTs are not expected to have the same consequences because HRTs do not change gene content or copy number while HNRTs lead to duplication and loss of genes that have been retained differently among homeologous segments or whose expression can be subfunctionalized (*e.g.*, ADAMS *et al.* 2004).

With that proviso, we estimate that the frequency of homeologous recombination is 10- to 100-fold increased during meiosis of *B. napus* haploids as compared to that of the natural or synthetic euploid *B. napus* (Table 4). Homeologous recombination also shows <10-fold reduction when compared to homologous recombination measured on the same intervals (Table 3). It must be emphasized here that the values of these different ratios have been computed using the frequency of homeologous recombination measured in *Darmor-bzh* haploids, which display high numbers of

bivalents at MI and have a permissive allele at the *PrBn* locus (JENCZEWSKI *et al.* 2003). These comparisons can then be readily interpreted to reflect the suppression of pairing competition between homologues and homeologues during meiosis of haploids and the overall reduction of recombination between divergent genomes. Differences in the magnitude of change in recombination frequency among linkage groups we examined provide considerably more information.

We observe only a slight reduction of homeologous *vs.* homologous recombination for most intervals and linkage groups (Table 3). This confirms that the A and C homeologous regions of *B. napus*, which diverged ~4 MYA (INABA and NISHIO 2002), remain very closely related. This assertion is in agreement with the fact that very few gene rearrangements differentiate the homeologous regions of *B. napus* (RANA *et al.* 2004). The slightest reduction is observed at opposing ends of N11, suggesting that these regions are the least divergent against their homeologues on N1. This assertion is not confirmed when looking at N1, which shows a lower relative proportion of homeologous recombinants (Table 3) and a lower number of HNRTs (Table 2). This asymmetry indicates either that a double-strand break is more frequently resolved by crossing over rather than by conversion on N11 than on N1 (*e.g.*, JEFFREYS and MAY 2004) or that plants carrying N11.N1 (T) are more viable than those carrying N1.N11 (T). Surprisingly, such an asymmetry is not observed for the other chromosomal rearrangements we have detected.

The increase of haploid *vs.* euploid homeologous recombination shows considerable variation among the three pairs of homeologous regions analyzed in this study (Table 4). Part of this variation is certainly due to the fact that Udall *et al.*'s data originate from a variable number of segregating populations, which can bias comparison and lead to underestimating the frequency of HNRTs in some regions/linkage groups as compared to others. Notably this could be the case for N9 for which UDALL *et al.* (2005) estimated the frequency of HNRTs using only two of four populations. Correcting for this bias, more than a twofold difference persists between linkage groups. This variation occurs even if the numbers of HNRTs detected for the three pairs of homeologous regions analyzed in this study are ranked in the same order as that in UDALL *et al.* (2005) (N1/N11 > N9/N18 ≫ bottom N3/N13). Specifically we observe no increase of haploid *vs.* euploid homeologous recombination at the bottom of N3/N13 that shows a constant very low number of rearrangements. We have confirmed that these regions are not prone to rearrange during haploid meiosis by detecting a similarly low amount of loss and duplication in two progenies derived from very different haploid genotypes (*Darmor-bzh* and *Yudal* haploids; Table 1). Characterization of the Y9H11 rearrangement indicates that the bottoms of N3 and N13 do not recombine with one another. Overall, these results show that a local disruption of synteny between otherwise extensively collinear chromosomes (PARKIN *et al.* 2003, 2005) is sufficient to preclude homeologous recombination in that rearranged region. Likewise, recombination data confirm that the homeologous regions between N9 and N18, which consist of juxtaposed genomic blocks from different Arabidopsis chromosomes (PARKIN *et al.* 2005) are slightly more divergent than the two arms of N1 and N11, which are collinear to a single region of Arabidopsis chromosomes, respectively. All these results suggest that the level of (macro)synteny can be used as a rough indicator of the likelihood of homeologous recombination occurring and give hints toward the recombination frequency (see N1/N11 asymmetry).

Does recombination occur between paralogous regions? Half the losses and duplications of homeologous loci detected in this study occurred independently. Although some of these rearrangements may have resulted from the disjunction of multivalents including homeologous chromosomes (see above), most of them do not result from recombination between regions of primary homeology. Several mechanisms have been described that generate losses and duplications: centric misdivision of univalents (*e.g.*, FRIEBE *et al.* 2005), unequal breakage of the anaphase bridge (*e.g.*, SIROKY *et al.* 2003), ectopic recombination between interspersed repeated sequences (*e.g.*, MONTGOMERY *et al.* 1991), and transposition (McCLINTOCK 1953; BENNETZEN 2000). Our FISH results point to a very appealing alternative

to these mechanisms, which echoes the highly replicated structure of the *B. napus* genome, *i.e.*, that recombination may occur between regions that show intragenomic (paralogues) or intergenomic homology (intergenomic homologues do not equal homeologues). Our FISH data show that autosyndesis is *quasi*-systematically observed at MI and represents 20% of the bivalents. These autosyndetic bivalents are likely to be chiasmatic (see above), indicating that recombination may occur between paralogous regions. However, paralogous regions seem to recombine less than homeologous regions; this is in agreement with the fact that paralogous regions within *B. oleracea* and *B. rapa* genomes diverged four to five times earlier than homeologous regions. Then, more gene rearrangements differentiated paralogous from homeologous regions (INABA and NISHIO 2002; RANA *et al.* 2004; TOWN *et al.* 2006; YANG *et al.* 2006).

We attempted to test for the occurrence of exchanges between regions showing intragenomic (and intergenomic) homology. Although we used a very dense genetic map with a mean and a maximum distance between adjacent markers of 5.5 and 28 cM, respectively (R. DELOURME, unpublished data), complete examination could not be achieved at all locations, due to the lack of markers with suitable polymorphisms (*e.g.*, N18 remained unexamined in the case of Y9H11) and/or the lack of accurate identification of all the paralogous regions. The most saturated marker survey was achieved for Y9H11 that showed concurrent loss and duplication of paralogous regions but revealed independent segregation of these regions; Y9H11 displayed a few multivalents at MI (10.5% of PMCs), suggesting that the missing part of N3 was not lost from a simple deletion but from a more complex chromosome reshuffling.

A surprising picture emerges from our data. Our study has shown that recombination occurs preferentially between homeologous regions and it is striking to observe that autosyndesis occurs more frequently within *B. napus* haploids than in monoploid *B. rapa* and *B. oleracea* in which autosyndesis was observed in <20 and 45% of PMCs, respectively (ARMSTRONG and KELLER 1981, 1982). Preferential homeologous pairing should effectively compete with autosyndesis and reduce, not increase, its level in *B. napus* haploids. This point deserves further consideration, notably by taking into account the variability of meiotic behaviors in the haploid forms of *B. napus* (JENCZEWSKI *et al.* 2003).

Concluding remarks: Numerous studies have now demonstrated that homeologous reciprocal and non-reciprocal translocations exist in the genome of *B. napus* (PARKIN *et al.* 1995; LOMBARD and DELOURME 2001; PIQUEMAL *et al.* 2005; UDALL *et al.* 2005). Yet, it is not known whether these rearrangements have occurred at the onset of *B. napus* polyploid formation or whether they have progressively accumulated during the subsequent generations (UDALL *et al.* 2005). Our study

provides strong evidence that duplication or loss of chromosomal regions occurs at high frequencies during meiosis of *B. napus* haploids. One may therefore envisage that *B. napus* haploids, which arise spontaneously in wild populations at a frequency up to 6/1000 (THOMPSON 1969; STRINGHAM and DOWNEY 1973), could serve as a recurrent source of chromosomal rearrangements. The *B. napus* haploid \times euploid cross could therefore be instrumental for both evolutionary and agricultural purposes by changing genomic structure (*e.g.*, duplication of regions of interest), and so this would generate new intraspecific variability on which natural or human selection can act (RAMANNA and JACOBSEN 2003; PIRES *et al.* 2004). More generally, our study points to the role that restituted gametes and sexual polyploidy can play in the genome evolution of nascent polyploids (RAMSEY and SCHEMSKE 2002). LUKENS *et al.* (2006) recently indicated that sequence loss occurred at very low frequency in the S_0 generation of colchicine-doubled and spontaneously doubled re-synthesized *B. napus* polyploids. It would be worthwhile to test if more numerous rearrangements are triggered when the S_0 -re-synthesized *B. napus* polyploids originate from restituted gametes produced by the $A \times C$ hybrids. Finally, the fact that homeologous recombination is sharply increased in haploids make these plants even more attractive models to gain insights into the genetic and genomic factors that influence the rate and pattern of intergenomic recombination. A key step will be to compare homeologous recombination frequencies between genotypes that have been shown to display different meiotic behaviors at MI (JENCZEWSKI *et al.* 2003).

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